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Effect of phenolic compounds on protein cross-linking and properties of film from fish myofibrillar protein

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ABSTRACT

The effects of several phenolic ocmpounds (caffeic acid, catechin, ferullic acid and tannic acid) at various concentrations (1, 3 and 5% based on protein) on cross-linking and properties of film from myofibrillar proteins of bigeye snapper (*Priacanthus tayenus*) were investigated. Among all phenolic compounds used, tannic acid exhibited the highest cross-linking ability on myofibrillar protein as evidenced by higher decrease in free amino groups with coincidentally lower band intensity of myosin heavy chain (MHC). In addition, the extent of protein cross-linking increased with increasing concentration of phenolic compounds. Addition of phenolic compounds could enhance mechanical properties of the resulting films. As phenolic compounds content increased, Young's modulus (E) and tensile strength (TS) of the films increased, while their elongation at break (EAB) decreased ($P < 0.05$), suggesting stronger and stiffer film structure. At the same concentration used, tannic acid rendered the film with higher mechanical properties, compared to others. Phenolic compounds decreased film transparency and affected color of the films differently, depending on types and concentrations used. Films from myofibrillar proteins with and without polyphenol generally had the excellent barrier properties to UV light at the wavelength of 200–800 nm. Therefore, it could potentially be used as inner packaging material for high-fat foods to prevent the lipid oxidation and thus prolonging the shelf-life of foods during storage.

1. Introduction

The non-biodegradability of most synthetic polymer-based packaging as well as the increasing environmental concern by consumers and government bodies have paved the way for alternative approaches [1]. This awareness has led to a focus on eco-friendly packaging materials derived from naturally occurring polymer in order to reduce environmental pollution and ecological related problems caused by non-biodegradable plastic packaging [2]. Currently, there has been an interest in edible film made from renewable and natural polymers such as proteins, polysaccharides and lipids. Among them, protein-based edible films are the most attractive due to impressive gas barrier properties, compared with those prepared from lipids and polysaccharides [3]. The properties of protein films are determined by their microstructure, which significantly varies depending on the protein structure and intermolecular interaction between polypeptide chains [4]. The functional properties of protein-based edible films are better than those of polysaccharide and fat-based films due to the unique structure of proteins (20 different monomers), which confer a wider range of functional properties, especially a high intermolecular binding potential [5]. It has been known that protein based-films have good oxygen, carbon dioxide and lipid barrier properties [6,7]. Protein-based film potentially used for coating or packaging could improve shelf-life and maintain the quality of foods during storage, by serving as selective barrier to moisture transfer, oxygen uptake, light transmission, losses of volatile aroma compounds [8]. Food packaging system using plastic packaging materials typically encounters several problems involving mass transfer phenomena; atmospheric oxygen penetration into foods causes oxidation of food ingredient; inks, solvents and monomeric additives in plastic packaging materials can migrate into foods. Therefore, biopolymer-based materials including protein films and coatings may wrap these food products or be located between heterogeneous parts of food products to prevent these migration phenomena and preserve food quality [9]. Protein-based films have been applied for coating nuts or used for bakery products [10].

Among protein from different sources, myofibrillar and sarcoplasmic proteins from fish muscle have been used as film forming material [11–15]. Thailand is one of the largest surimi producers in Southeast Asia. Bigeye snapper (*Priacanthus* spp.) is one of fish species commonly used for surimi production owing to its excellent gel-forming ability [11]. Apart from gelation, the appropriate development of myofibrillar protein film from bigeye snapper muscle

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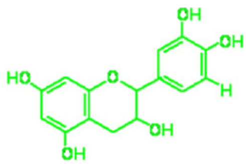
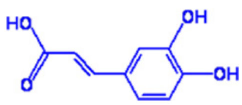
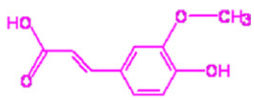
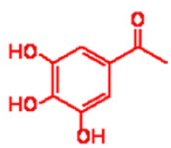
Table 1
Molecular structures and some physical and chemical properties of phenolic compounds used in this study.

Phenolic compounds	Molecular structure	Physical state	Solubility in water	Melting temp. (°C)	Acidity (pK_a)
Catechin		Yellowish crystals	1.76–2.75 g/L	177	8.45–8.91
Caffeic acid		Yellowish crystal	Soluble in hot water (soluble in alcohol)	223–225	8.69–8.69
Ferulic acid		Light yellowish solid powder	Soluble in cold water 0.9 g/L	168–172	4.52–9.36
Tannic acid		Yellowish brown powder	2850 g/L	200–210	ca. 10
	where, G = 				

should be an alternative promising means to obtain the nutritional and biodegradable film. Chinabhark [15] prepared films from bigeye snapper, threadfin bream and goat fish surimi plasticized with 50% glycerol based on protein, and found that films from bigeye snapper exhibited the highest tensile strength and elongation at break compared to those of threadfin bream and goat fish. Chinabhark et al. [11] reported that bigeye snapper myofibrillar protein films prepared from film-forming solution (FFS) containing protein of 2% (w/v) at acidic pH (pH = 3) had better mechanical properties than those prepared from 1% protein FFS at alkaline pH (pH = 11). However, the poor mechanical properties of protein-based films especially when exposing to wet and humid conditions as well as the higher cost of protein compared to conventional oil-based polymers limit its application in food packaging [13–16]. Hence, the structure modifications of protein are required to enhance its mechanical strength and water-resistant properties. In order to improve the properties of protein-based film, different approaches have been used including chemical, physical and enzymatic modifications [17]. Chemical cross-linking of natural proteins has a long history in the development of protein-based materials for food processing, packaging, coating formulations and pharmaceutical/medical applications [18].

The study of phenol–protein interactions has been carried out for more than half a century. Phenolic compounds derived from various sources have been reported to be interactive or reactive with proteins, and resulted in improved gel or film properties for gelatin-based materials [19]. Polyphenols are known to react under oxidizing conditions with side chain amino groups of peptides, leading to the formation of protein cross-links [20]. Ferulic acid can cross-link with protein and polysaccharides by producing a resonance-stabilized free radical intermediate [21]. Oxidized ferulic acid can react with amino and thiol groups in protein [22]. Additionally, free radical formed from ferulic acid can react with tyrosine and with itself to form diferulic acid, which acts as a bridge between protein molecules [22]. Tannic acid also showed the ability to bind proteins [23]. Covalent bonds between phenolic compounds and proteins yielded the cross-links, which are more rigid and thermally stable than other interactions. Nevertheless, information regarding the effect of phenolic compounds on cross-linking of fish myofibrillar protein (FMP) and on its film properties is very scarce. Thus, the study aimed to investigate the effect of different phenolic compounds, including ferulic acid, tannic acid, catechin and caffeic acid on the properties of films from bigeye snapper (*Priacanthus tayenus*) muscle proteins.

2. Materials and methods

2.1. Chemicals

High molecular weight protein markers, 2,4,6-trinitrobenzenesulfonic acid (TNBS), 5,5′-dithiobis (2-nitrobenzoic acid) (DTNB), ferulic acid, tannic acid and β-mercaptoethanol (β-ME) were obtained from Sigma (St. Louis, MO, USA). Caffeic acid and catechin were purchased from Fluka (Buchs, Switzerland). Glycerol and tris (hydroxymethyl) aminomethane were obtained from Merck (Darmstadt, Germany). Sodium dodecyl sulfate (SDS), Coomassie Blue R-250 and *N*,*N*,*N*′,*N*′-tetramethylethylenediamine (TEMED) were procured from Bio-Rad Laboratories (Hercules, CA, USA). All chemicals were of analytical grade. Table 1 shows molecular structures as well as some physical and chemical properties of phenolic compounds used in this study.

2.2. Fish sample

Bigeye snapper (*P. tayenus*) was used as source of myofibrillar protein for film preparation. Bigeye snapper is white muscle fish species which is in huge quantity on the market in Southern Thailand. This fish also has lower price compared to other white muscle fish species and it is mainly used in surimi industry. Moreover, this fish is particularly rich in protein, typically containing about 93–96% protein (dry-basis weight). Like other white muscle fish species, bigeye sanapper muscle possesses a large amount